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(54) Title: ONCOLOGY DRUG INNOVATION

(57) Abstract

The present invention describes methods for identification of molecules expressed at a different level on the cell surface of cancer cells compared to non-malignant cells and methods of identification of cancer specific promoters to be used singly or in combination for delivery and expression of therapeutic genes for treatment of cancer. The invention furthermore describes targeting complexes targeted to cell surface molecules identified by the methods of the invention. In embodiments of the invention said targeting complexes comprise the promoters identified by the methods of the invention. In addition the invention describes methods of identifying binding partners for the cell surface molecules and the binding partners per se. Methods of treatment using the targeting complexes and uses of the targeting complexes for the preparation of a medicament are also disclosed by the invention. Furthermore, the invention describes uses of the cell surface molecules or fragments thereof for preparation of vaccines.

Description Claims

Oncology drug innovation Field of invention The present invention relates to methods of identification of molecules on the cell surface of cancer cells and a method of identification of cancer specific promoters to be used singly or in combination for delivery and expression of therapeutic genes for treatment of cancer.

Background of the invention Approximately half of all patients with cancer have disseminated disease at the time of diagnosis. Existing cancer therapies are able to cure only 5-7% of these patients.

Consequently, there is a great need for more effective drugs, which can be admin- istered systemically alone or in combination with existing treatments. Methods util- ising gene therapy to deliver efficient and specific treatment of cancer cells is there- fore a promising strategy. However, strategies applied to this date have only had limited success and the development of suitable delivery systems need further de- velopment.

Delivery vectors The choice of the delivery vector for gene therapy is a major issue. Many vector systems have been tested for their suitability for gene transfer, including viral vec- tors such as retrovirus, adenovirus, adeno-associated virus, lentivirus and non viral vectors such as complexing with liposomes, cationic lipids or polycations. However, all of these vectors have specific advantages and limitations. Retrovirus requires mitotic division for transduction, but mediate long term expression, as they integrate in the genome. Adenovirus will transduce both dividing and non-dividing cells, but only transiently as they remain episomal. Adenovirus, however, are highly immuno- genic and retrovirus are rapidly inactivated by the human complement system. Len- tivirus does not induce immune response, but involve specific safety concerns, as it is a member of the immunodeficiency virus. More than 75% of all protocols so far have used viral vectors despite these are difficult and expensive to produce, there is a

limited insert size of the therapeutic gene and there are many safety considera- tions to be made. Therefore, the majority of the protocols used for adenoviral vec- tors have administered the therapeutic gene by local delivery (injection into the tu- mour) to increase the local titer of the virus and avoid immunogenic response, but even the highest titer system has not yet been sufficient to cure local tumours. A major disadvantage of viral vector systems is that their uptake is unspecific and not targeted to the cancer cells. However, as adenovirus still is the preferred vector due to its efficiency of delivery, ways of reducing the immune response and target the virus to specific cells are under development. On the other hand, liposomes and polycation complexes, which are less immunogenic, easier to produce and do not need the safety considerations of viral vectors have much lower transfection effi- ciency than viral transduction and also lack the cell specificity. However, polycations have the ability to compact and neutralise the charge of the delivered DNA and PEI complexes appear relatively stable in the blood system (Goula et al., 1998; reviewed in Mountain, 2000).

To assure high specificity and to limit undesired side effects of the treatment, it is of importance to design a vector or vehicle, which targets and delivers the therapeutic gene in question to the cancer cells efficiently and with high specificity. However, as described below, this involves assembly of a multi component vector.

Receptor targeting.

Functional receptors or other cell surface molecules, which can internalise by ligand or antibody binding on the cancer cell surfaces, can be used to target the gene de- livery to the cells. Receptor targeted gene delivery by means of DNA conjugated to a ligand of the receptor offers a promising approach. The major advantages of tar- geted gene delivery are that receptor targeting can be performed without virus, thus eliminating many of the obstacles present in current strategies of gene therapy.

Successful deliverance of genes to cancer cells using receptor targeting has been reported to a variety of different surface receptors including receptors for epidermal growth factor (Cristano and Roth, 1996, Frederiksen et al., 2000), folate (Gottschalk et al., 1994), transferrin (Wagner et al., 1990). High expression of a specific recep- tor is not always a pre-requisite for efficient receptor mediated uptake, as has been demonstrated for the epidermal growth factor receptor (Frederiksen et al., 2000).

However, many of the receptors expressed by cancer cells are also expressed by normal cells to some extent, meaning that normal cells will often be targeted as well.

This issue emphasises the need for further requirements for specificity for the ex- pression or nature of the therapeutic gene.

Molecular conjugates For targeted gene therapy it is essential that the ligand to be internalised and DNA expressing the therapeutic gene are physically associated for receptor mediated uptake. Several methods have been used for preparing non-viral, synthetic vectors of targeted DNA molecular conjugates by associating cationic polymers, such as poly-L-lysine (Frederiksen et al., 2000) or polyethylenimine (PEI) (Kircheis et al., 1997) (polyplexes) with the ligand and DNA. Successful gene targeting has been reported for a number of molecular conjugates. The ligand has either been cova- lently linked to the polycation, or biotinylated ligand and polylysin were complexed via streptavidine to form condensed conjugates with DNA, which are internalised by the receptor of the ligand. One of the advantages of these system over virus medi- ated transfer is the lack of size limitation of the DNA. PEI complexes, in addition, appear to be able to pass the capillary barrier in lung, making this compound one agent for molecular conjugates.

Endosomal release of molecular conjugate.

After endocytosis of the DNA/ligand conjugate by the receptor, the normal pathway would lead to degradation and loss of DNA. It has therefore proven essential to in- clude an endosomolytic agent in the molecular conjugate. Adenovirus, replication deficient adenovirus and the viral capside have all proven to be very efficient for endosomal lysis, when included in the molecular conjugate. However, all the reser- vations of unspecific uptake, safety and immunogenic response applying to use of using adenovirus as vectors also apply for this system. Inclusion of other fusogenic peptides containing amino acid sequences from e. g. influenza virus, toxins or syn- thetic peptides in the molecular conjugate have been tested for cytoplasmic release.

These have the advantage of less immunogenicity and lower cost, but have been shown to be less effective in endosomal lysis than adenovirus. However, if the mo- lecular conjugate is formed using the polycationic PEI, inclusion of endosomolytic agents are not necessary, as PEI has an intrinsic endosome-buffering capacity re- sulting in endosomal swelling and rupture.

Cancer specific promoters An increase in the specificity of the targeting of a therapeutic gene to cancer cells can be obtained if a tumour specific promoter controlling the expression can be used (reviewed in Nettelbeck et al., 2000). Promoters for genes, whose expression is specific for the malignant phenotype, but show no tissue specificity such as telomer- ase have been used. Also, promoters regulating oncofetal antigens, which are not normally expressed in the adult, have been found to be active in tumor cells, such as carcinoembryonic antigen (CEA). However, the activity of these promoters (com- pared to strong, constitutive active viral promoters) have often proven not to mediate sufficient expression of the therapeutic gene, wherefore the tumour specific genes have been used for activation of another, stronger promoter controlling the thera- peutic gene. Another disadvantage of oncofetal promoters is that these promoters will only be active in a subset of tumour types, depending on the tissue origin of the tumour. Alternatively, synthetic promoters have been designed taking advantage of the fact that many oncogenes which are overexpressed in cancer cells are tran- scription factors, which can mediate high transcriptional activity from their respective DNA recognition sequences.

Therapeutic genes The product of a therapeutic gene must be able to effectively induce cell death.

Gene therapy strategies for cancer treatment have used many different approaches.

These include immunogene therapy such as cytokine stimulation of immune system (enhancing the immune response against tumour cells), selective prodrug activation, suicide genes, restoration of tumor suppressor genes and inhibition of activated on- cogenes (reviewed in Frederiksen et al., 1999; Gunji et al., 2000). Indeed, most of the present therapeutic protocols in clinical trials against cancer involve immuno- therapy. However, as the molecular phenotype of many types of cancer regarding aberrant expression or mutations of oncogenes and tumour suppressor genes, these are obvious candidates to target. Therapeutic gene products reducing expres- sion or activity of oncogenes, such as antisense RNA or neutralising antibody frag- ments, have been tried and shown to inhibit proliferation. However, oncogene inacti- vation does not necessarily kill the cells and is therefore probably not applicable for short term treatment. One of the at present promising strategies is to reintroduce tumour suppressor genes, as most cancer cells exhibit loss of function of one or more of these genes. Of particular interest is the tumour suppressor gene TP53 en- coding p53, which is a transcription factor, which activates genes known to be in- volved in cell cycle arrest and induction of apoptosis. Reintroduction of wild type p53 has been shown to markedly reduce tumour cell growth or induce apoptosis of can- cer cells in both in vitro and in vivo systems (Roth et al., 1996; Nielsen and Maneval, 1998).

However, gene products rendering cells sensitive to otherwise harmless drugs has also been extensively used for gene therapy trials. In particular, the herpes simplex virus thymidine kinase (HSV-tk) in combination with the nucleoside analogue drug gangcyclovir has been used. However, the conversion of the drug to a toxic nucleo- side analogue by the enzyme only will kill cells, which are dividing. However, the toxic products are transmitted to surrounding cells by the so-called"by-stander"ef- fect, making the approach potential for systems with low targeting efficiency.

Summary of the invention Accordingly, it is a first objective of the present invention to provide methods for identifying a plurality of cell surface molecules, which are expressed at a different level in malignant cells compared with normal cells, comprising the steps of: i) Providing at least 3 malignant cell lines selected from the group consisting of CPH 54 A, CPH 54 B, GLC 2, GLC 3, GLC 14, GLC 16, GLC 19, GLC 26, GLC 28, DMS 53, DMS 79, DMS 92, DMS 114, DMS 153, DMS 273, DMS 406, DMS 456, NCI H69, NCI N417, MAR H24, MAR 86 MI, SHP-77, NCI-H2171, NCI-H2195, NCI-H2196, NCI-H2198, NCI-H2227, NCI-H2286, NCI-H2330, NCI-H735, NCI-H1339, NCI-H1963, NCI-H2107, NCI-H2108, NCI-H1304, NCI-H1341, NCI- H1417, NCI-H1436, NCI-H1522, NCI-H1618, NCI-H1672, NCI- H1694, NCI-H1836, NCI-H1870, NCI-H1876, NCI-H1882, NCI- H1926, NCI-H1930, NCI-H1994, NCI-H2029, NCI-H2059, NCI- H2066, NCI-H2081, NCI-H2141, NCI-H211, NCI-H220, NCI-H250,

 NCI-H524, NCI-H592, NCI-H711, NCI-H719, NCI-H740, NCI-H748,

 NCI-H774, NCI-H841, NCI-H847, NCI-H865, NCI-H1048, NCI-H1059,

 NCI-H1092, NCI-H1105, NCI-H1184, NCI-H1238, NCI-H1284, NCI- H1688, NCI-H187, NCI-H378, NCI-H526, NCI-H660, NCI-H889, NCI- H60, NCI-H196, NCI-H446, NCI-H209, NCI-H146, NCI-H82, NCI- H460, NCI-H345, NCI-H510A, NCI-128, NCI-446, SW 1271 ii) Providing at least 3 total RNA samples derived from normal tissue selected from the group consisting of liver, heart, kidney, lung, adrenal gland, colon, pancreas, small intestine, spleen, skeletal muscle, trachea, prostate, placenta, salivary gland, testes, leucocytes, leucocytes, brain, adipose tissue, bladder, breast, cervix, esophagus, larynx, ovary, rectum, skin, spinal cord, stomach, thymus, thyroid and uterus. iii) Comparing the expression of mRNA in the cell lines according to step i) and tissue samples according to step ii) iv) Identifying nucleic acid sequences, wherein a) there is a difference between the amount of mRNA expressed in one or more cell lines according to i) and the amount of mRNA expressed in one or more tissues according to ii); and/or b) there is essentially no difference in the amount of mRNA expressed in at least two cell lines according to i); and/or c) there is essentially no difference in the amount of mRNA expressed in at least two tissue samples according to ii); and v) Selecting among the nucleic acid sequences according to iv), nucleic acid sequences encoding for potential cell surface molecules.

It is a second objective of the present invention to provide methods of identifying first nucleic acid sequences, which are capable of directing expression of second nucleic acid sequences operably linked thereto, wherein the level of said expression is different in malignant cells compared with normal cells comprising the steps of: i) Providing at least 3 malignant cell lines selected from the group consisting of CPH 54 A, CPH 54 B, GLC 2, GLC 3, GLC 14, GLC 16, GLC 19, GLC 26, GLC 28, DMS 53, DMS 79, DMS 92, DMS 114, DMS 153, DMS 273, DMS 406, DMS 456, NCI H69, NCI N417, MAR H24, MAR 86 MI, SHP-77, NCI-H2171, NCI-H2195, NCI-H2196, NCI- H2198, NCI-H2227, NCI-H2286, NCI-H2330, NCI-H735, NCI-H1339, NCI-H1963, NCI-H2107, NCI-H2108, NCI-H1304, NCI-H1341, NCI- H1417, NCI-H1436, NCI-H1522, NCI-H1618, NCI-H1672, NCI- H1694, NCI-H1836, NCI-H1870, NCI-H1876, NCI-H1882, NCI- H1926, NCI-H1930, NCI-H1994, NCI-H2029, NCI-H2059, NCI- H2066, NCI-H2081, NCI-H2141, NCI-H211, NCI-H220, NCI-H250, NCI-H524, NCI-H592, NCI-H711, NCI-H719, NCI-H740, NCI-H748,

 NCI-H774, NCI-H841, NCI-H847, NCI-H865, NCI-H1048, NCI-H1059,

 NCI-H1092, NCI-H1105, NCI-H1184, NCI-H1238, NCI-H1284, NCI-

 H1688, NCI-H187, NCI-H378, NCI-H526, NCI-H660, NCI-H889, NCI- H60, NCI-H196, NCI-H446, NCI-H209, NCI-H146, NCI-H82, NCI- H460, NCI-H345, NCI-H510A, NCI-128, NCI-446, SW

1271 ii) Providing at least 3 RNA samples derived from normal tissue samples derived from the group consisting of liver, heart, kidney, lung, adrenal gland, colon, pancreas, small intestine, spleen, skeletal muscle, trachea, prostate, placenta, salivary gland, testes, leucocytes, brain, adipose tissue, bladder, breast, cervix, esophagus, larynx, ovary, rectum, skin, spinal cord, stomach, thymus, thyroid and uterus. iii) Comparing the expression of mRNA in the cell lines according to i) and tissue samples according to ii) iv) Identifying second nucleic acid sequences, wherein a) there is a difference between the amount of mRNA expressed in one or more cell lines according to i) and the amount of mRNA expressed in one or more tissues according to ii); and/or b) there is essentially no difference in the amount of mRNA expressed in at least two cell lines according to i); and/or c) there is essentially no difference in the amount of mRNA expressed in at least two tissue samples according to ii) v) Identifying first nucleic acid sequences operably linked to the second nucleotide sequences identified in step iv) It is a third objective of the present invention to provide uses of a pharmaceutically effective amount of the cell surface molecules identified according to the present invention for the preparation of a vaccine. Furthermore, the present invention pro- vides uses of a pharmaceutical effective amount of a nucleic acid sequence en- coding a cell surface molecule identified according to the methods of the present invention for the preparation of a vaccine. The present invention also provides uses of a pharmaceutically effective amount of a cell surface molecule and/or a nucleic acid sequence encoding such a cell surface molecule for the preparation of a vac- cine, wherein said cell surface molecule preferably comprises or essentially consists of or for example is GRIA2, such as LPR8, for example is CHRNA5, such as TMEFF, for example is NPTXR, such asTransferrin receptor; such as type 11 membrane protein clone : for example is HP10481 ; such as type 11 membrane protein clone : such as HP10390 ; for example is PG40; such as TRC8; for example is TR2- 11; such as OA3 antigenic surface determinant; for example is integrin alpha 6, For example GPllb ; such as vitronectin receptor alpha subunit; for example is integrin alpha-7 ; such as integrin alpha E precursor; for example is integrin alpha 6B; such as integrin alpha 5 subunit; for example is integrin beta-5 subunit; such as integrin alpha-3 chain; for example is RYK; such as amyloid precursor protein-binding pro- tein 1; for example is putative transmembrane GTPase; such as membrane cofactor protein; FOR EXAMPLE GLVR1; for example is Mr 110,000 antigen; for example is syndecan-1; such as putative seven transmembrane domain protein; for example is LCA-homolog/LAR protein; such as M6 antigen; for example is Me491/CD63 anti- gen; such as multispanning membrane protein; for example is DDR; such as autocrine motility factor receptor; for example is insulin receptor precursor; such as IGF1 R, for example is insulin-like growth factor 11 receptor; such as SAS; for exam- ple is TAPA-1; such as MICB ; for example is MHC class II HLA-DR7-associated glycoprotein beta-chain; such as HLA-DP; for example is bone small proteoglycan I biglycan ; such as CAR; for example is MEA11 ; such as interferon-gamma receptor alpha chain; for example is Polymeric immunoglobulin receptor; such as metabo- tropic glutamate receptor type 4; for example is metabotropic glutamate receptor 8; such as CLPTM1; for example is MAGE-4b; such as MAGE5a; for example is MAGE-3; such as MAGE-1; for example is MAGE6; such as MAGE-9; for example is MAGE11 ; such as CD24; for example is CD59; such as CD44; for example is low density lipoprotein receptor ; such as very low density lipoprotein receptor; for exam- ple is N-CAM; such as lamin B receptor homolog TM7SF2; for example is putative T1/ST2 receptor binding protein precursor; such as NTR2 receptor; for example is RAGE-4; such as HLA-G1 ; for example is MOAT-C; such as alpha 2 delta calcium channel subunit isoform 1 ; for example is LFA-3; such as L1-CAM ; for example is AVPR2; such as C1 p115 C1 ; for example is TE2; such as RbP; for example is HCF1; such as IRAK ; for example is CD151 ; such as surface antigen; for example is MAG; such as GPR19 ; for example is pcta-1; such as PRAME; for example is vaso- pressin activated calcium mobilizing receptor-like protein; such as serotonin receptor 5-HT4B; for example is serotonin. 1 D receptor- (5-HT1 D-) ; such as CD9; for example is LDL receptor member LR3; such as DR6; for example is tumor necrosis factor receptor; such as HG38; for example is urokinase-type plasminogen receptor; such as FGF receptor; for example is nerve growth factor receptor; such as cys- tine/glutamate transporter; for example is-CB1 cannabinoid receptor (CNR1) ; such as PSG; for example is PSG13' ; such as CPE-receptor; for example is CRH2R; such as OCI5 ; for example is TRAIL receptor 2; such as

HNMP-1; for example is kidney alpha-2-adrenergic receptor; such as erythropoietin receptor; for example is chondroitin sulphate proteoglycan versican V1 ; for example is mGluR1beta ; such as CD97; for example is L6; such as NY-ESO-1; for example is T-cell receptor alpha delta ; such as ror1 ; for example is ror2; such as SSTR2 ; for example is VESPR; such as IgG Fc receptor; for example is glutamate receptor subunit GluRC ; such as HEK2; for example. is PVR; such as CEA; for example is CC-chemokine-binding receptor JAB61; such as HER2; for example is HER3 ; such as hypothetical protein FLJ22357 similar to Epidermal growth factor receptor-related protein; for example is putative endothelin receptor type B-like protein; such as GLVR2; for example is P2X4 purinoreceptor; such as FPRL1; for example is Atrial natriuretic peptide clear- ance receptor; for example is gastrin/CCK-B receptor; such as Neuromedin B re- ceptor; for example is GFRA3; such as GRPR; for example is CDH1; such as CDH2; for example is TGFBR1; such as TGFBR2; for example is TGFBR3; such as precursor of epidermal growth factor receptor.

It is a fourth objective of the present invention to provide uses of a cell surface molecule identified according to the methods described by the present invention as a drug target, wherein said drug target is capable of binding a binding partner and internalising said binding partner into cells expressing said cell surface molecule.

Furthermore, the present invention provides uses of a cell surface molecule which preferably comprises or essentially consists of or for example is GRIA2, such as LPR8, for example is CHRNA5, such as TMEFF, for example is NPTXR, such as Transferrin receptor; such as type 11 membrane protein clone : for example is HP10481 ; such as type 11 membrane protein clone : such as HP10390 ; for example is PG40; such as TRC8; for example is TR2-11 ; such as OA3 antigenic surface determinant; for example is integrin alpha 6, For example GPllb ; such as vitronectin receptor alpha subunit; for example is integrin alpha-7 ; such as integrin alpha E precursor; for example is integrin alpha 6B; such as integrin alpha 5 subunit; for example is integrin beta-5 subunit; such as integrin alpha-3 chain; for example is RYK; such as amyloid precursor protein-binding protein 1; for example is putative transmembrane GTPase ; such as membrane cofactor protein; FOR EXAMPLE GLVR1; for example is Mr 110,000 antigen; for example is syndecan-1; such as putative seven transmembrane domain protein; for example is LCA-homolog/LAR protein; such as M6 antigen; for example is Me491/CD63 antigen; such as multispanning membrane protein; for example is DDR; such as autocrine motility factor receptor; for example is insulin receptor precursor; such as IGF1 R, for example is insulin-like growth factor 11 receptor; such as SAS; for example is TAPA- 1; such as MICB ; for example is MHC class 11 HLA-DR7-associated glycoprotein beta-chain; such as HLA-DP; for example is bone small proteoglycan I biglycan ; such as CAR; for example is MEA11 ; such as interferon-gamma receptor alpha chain; for example is Polymeric immunoglobulin receptor; such as metabotropic glutamate receptor type 4; for example is metabotropic glutamate receptor 8; such as CLPTM1; for example is MAGE-4b; such as MAGE5a ; for example is MAGE-3; such as MAGE-1; for example is MAGE6; such as MAGE-9; for example is MAGE11 ; such as CD24; for example is CD59; such as CD44; for example is low density lipoprotein receptor; such as very low density lipoprotein receptor; for example is N-CAM; such as lamin B receptor homolog TM7SF2; for example is putative T1/ST2 receptor binding protein precursor; such as NTR2 receptor; for example is RAGE-4; such as HLA-G1 ; for example is MOAT-C; such as alpha 2 delta calcium channel subunit isoform I ; for example is LFA-3; such as L1-CAM ; for example is AVPR2; such as C1 p115 C1 ; for example is TE2; such as RbP; for example is HCF1; such as IRAK; for example is CD151 ; such as surface antigen; for example is MAG; such as GPR19; for example is pcta-1; such as PRAME; for example is vasopressin activated calcium mobilizing receptor-like protein; such as serotonin receptor 5-HT4B; for example is serotonin 1 D receptor (5-HT1 D-) ; such as CD9; for example is LDL receptor member LR3; such as DR6; for example is tumor necrosis factor receptor; such as HG38; for example is urokinase-type plasminogen receptor; such as FGF receptor; for example is nerve growth factor receptor; such as cystine/glutamate transporter; for example is CB1 cannabinoid receptor (CNR1) ; such as PSG; for example is PSG13' ; such as CPE-receptor; for example is CRH2R;

such as OC15 ; for example is TRAIL receptor 2; such as HNMP- 1; for example is kidney alpha-2-adrenergic receptor ; such as erythropoietin receptor; for example is chondroitin sulphate proteoglycan versican V1 ; for example is mGluR1 beta ; such as CD97; for example is L6; such as NY-ESO-1; for example is T-cell receptor alpha delta ; such as ror1 ; for example is ror2; such as SSTR2; for example is VESPR; such as IgG Fc receptor; for example is glutamate receptor subunit GluRC ; such as HEK2; for example is PVR; such as CEA; for example is CC-chemokine-binding receptor JAB61; such as HER2; for example is HER3 ; such as hypothetical protein FLJ22357 similar to Epidermal growth factor receptor-related protein; for example is putative endothelin receptor type B-like protein; such as GLVR2; for example is P2X4 purinoreceptor; such as FPRL1; for example is Atrial natriuretic peptide clearance receptor; for example is gastrin/CCK-B receptor; such as Neuromedin B receptor; for example is GFRA3; such as GRPR; for example is CDH1; such as CDH2; for example is TGFBR1; such as TGFBR2; for example is TGFBR3; such as precursor of epidermal growth factor receptor as drug target, wherein said drug target is capable of binding a binding partner and internalising said binding partner into cells expressing said cell surface molecule.

It is a fifth objective of the present invention to provide methods of identifying and/or preparing specific binding partners comprising the steps of i) Providing a cell surface molecule identified by the methods described by the present invention ii) Identifying and/or preparing binding partners capable of associating with said cell surface molecules It is furthermore an objective of the present invention to provide methods of identifying and/or preparing specific binding partners comprising the steps of i) Providing a cell surface molecule which preferably comprises or essentially consists of or for example is Transferrin receptor; such as type 11 membrane protein clone: for example is HP10481; such as type 11 membrane protein clone : such as HP10390 ; for example is PG40; such as TRC8 ; for example is TR2-11 ; such as OA3 antigenic surface determinant; for example is integrin alpha 6, For example GPllb ; such as vitronectin receptor alpha subunit; for example is integrin alpha-7 ; such as integrin alpha E precursor; for example is integrin alpha 6B; such as integrin alpha 5 subunit; for example is integrin beta-5 subunit; such as integrin alpha-3 chain; for example is RYK; such as amyloid precursor protein-binding protein 1; for example is putative transmembrane GTPase; such as membrane cofactor protein; FOR EXAMPLE GLVR1; for example is Mr 110,000 antigen; for example is syndecan-1; such as putative seven transmembrane domain protein; for example is LCA-homolog/LAR protein; such as M6 antigen; for example is Me491/CD63 antigen; such as multispanning membrane protein; for example is DDR; such as autocrine motility factor receptor; for example is insulin receptor precursor; such as IGF1 R, for example is insulin-like growth factor 11 receptor; such as SAS; for example is TAPA-1; such as MICB ; for example is MHC class 11 HLA-DR7-associated glycoprotein beta- chain; such as HLA-DP; for example is bone small proteoglycan I biglycan ; such as CAR; for example is MEA11 ; such as interferon- gamma receptor alpha chain; for example is Polymeric immunoglobulin receptor; such as metabotropic glutamate receptor type 4; for example is metabotropic glutamate receptor 8; such as CLPTM1; for example is MAGE-4b; such as MAGE5a ; for example is MAGE-3; such as MAGE-1; for example is MAGE6; such as MAGE-9; for example is MAGE11 ; such as CD24; for example is CD59; such as CD44; for example is low density lipoprotein receptor; such as very low density lipoprotein receptor; for example is N-CAM; such as lamin B receptor homolog TM7SF2; for example is putative T1/ST2 receptor binding protein precursor; such as NTR2 receptor; for example is RAGE-4; such as HLA-G1 ; for example is MOAT-C; such as alpha 2 delta calcium channel subunit isoform I ; for example is LFA-3; such as L1-CAM ; for example is AVPR2; such as C1 p115 C1 ; for example is TE2; such as RbP; for example is HCF1; such as IRAK; for example is CD151 ; such as surface antigen; for example is MAG; such as GPR19 ; for example is pcta-1; such as PRAME; for example is vasopressin activated calcium mobilizing receptor-like protein ; such as serotonin receptor 5-HT4B; for example is serotonin 1 D receptor (5-HT1 D-) ; such as CD9; for example is LDL receptor member LR3; such as DR6; for example is tumor necrosis factor receptor; such as HG38; for example is urokinase-type plasminogen receptor; such as FGF receptor ; for example is nerve growth factor receptor; such as cystine/glutamate transporter; for example is CB1 cannabinoid receptor (CNR1) ; such as PSG; for

example is PSG13' ; such as CPE- receptor; for example is CRH2R; such as OC15 ; for example is TRAIL receptor 2; such as HNMP-1; for example is kidney alpha-2- adrenergic receptor; such as. erythropoietin receptor; for example is chondroitin sulphate proteoglycan versican V1 ; for example is mGluR1 beta ; such as CD97; for example is L6; such as NY-ESO-1; for example is T-cell receptor alpha delta ; such as ror1 ; for example is ror2; such as SSTR2; for example is VESPR; such as IgG Fc receptor ; for example is glutamate receptor subunit GluRC ; such as HEK2 ; for example is PVR; such as CEA; for example is CC- chemokine-binding receptor JAB61; such as HER2; for example is HER3 ; such as hypothetical protein FLJ22357 similar to Epidermal growth factor receptor-related protein; for example is putative endothelin receptor type B-like protein; such as GLVR2; for example is P2X4 purinoreceptor; such as FPRL1; for example is Atrial natriuretic peptide clearance receptor; for example is gastrin/CCK-B receptor; such as Neuromedin B receptor; for example is GFRA3 ; such as GRPR ; for example is CDH1 ; such as CDH2; for example is TGFBR1; such as TGFBR2; for example is TGFBR3; such as precursor of epidermal growth factor receptor. ii) Identifying and/or preparing binding partners capable of associating with said cell surface molecules.

A further objective of the present invention is to provide isolated and/or purified specific binding partners capable of associating with cell surface molecules, which are expressed at a different level in malignant cells compared with normal cells, identified by the methods provided by the present invention. The present invention also provides isolated and/or purified specific binding partners capable of associating with a cell surface molecule which preferably comprises or essentially consists of or for example is GRIA2, such as LPR8, for example is CHRNA5, such as TMEFF, for example is NPTXR, such as Transferrin receptor; such as type 11 membrane protein clone : for example is HP10481; such as type 11 membrane protein clone : such as HP10390 ; for example is PG40; such as TRC8; for example is TR2-11 ; such as OA3 antigenic surface determinant; for example is integrin alpha 6, For example GPIIb ; such as vitronectin receptor alpha subunit; for example is integrin alpha-7 ; such as integrin alpha E precursor; for example is integrin alpha 6B; such as integrin alpha 5 subunit; for example is integrin beta-5 subunit; such as integrin alpha-3 chain; for example is RYK; such as amyloid precursor protein- binding protein 1; for example is putative transmembrane GTPase; such as membrane cofactor protein; FOR EXAMPLE GLVR1; for example is Mr 110,000 antigen; for example is syndecan-1; such as putative seven transmembrane domain protein; for example is LCA-homolog/LAR protein; such as M6 antigen; for example is Me491/CD63 antigen; such as multispanning membrane protein; for example is DDR; such as autocrine motility factor receptor; for example is insulin receptor precursor; such as IGF1 R, for example is insulin-like growth factor 11 receptor; such as SAS; for example is TAPA-1; such as MICB ; for example is MHC class 11 HLA- DR7-associated glycoprotein beta-chain; such as HLA-DP; for example is bone small proteoglycan I biglycan ; such as CAR; for example is MEA11 ; such as interferon-gamma receptor alpha chain; for example is Polymeric immunoglobulin receptor; such as metabotropic glutamate receptor type 4; for example is metabotropic glutamate receptor 8; such as CLPTM1; for example is MAGE-4b; such as MAGE5a; for example is MAGE-3; such as MAGE-1; for example is MAGE6 ; such as MAGE-9; for example is MAGE11 ; such as CD24; for example is CD59; such as CD44; for example is low density lipoprotein receptor; such as very low density lipoprotein receptor; for example is N-CAM; such as lamin B receptor homolog TM7SF2; for example is putative T1/ST2 receptor binding protein precursor; such as NTR2 receptor; for example is RAGE-4; such as HLA-G1 ; for example is MOAT-C; such as alpha 2 delta calcium channel subunit isoform I ; for example is LFA-3; such as L1-CAM ; for example is AVPR2; such as C1 p115 C1 ; for example is TE2; such as RbP; for example is HCF1; such as IRAK ; for example is CD151 ; such as surface antigen; for example is MAG; such as GPR19 ; for example is pcta-1; such as PRAME ; for example is vasopressin activated calcium mobilizing receptor-like protein; such as serotonin receptor 5-HT4B; for example is serotonin 1 D receptor (5-HT1 D-) ; such as CD9; for example is LDL receptor member LR3; such as DR6; for example is tumor necrosis factor receptor; such as HG38; for example is urokinase-type plasminogen receptor; such as FGF receptor; for example is nerve growth factor receptor; such as cystine/glutamate transporter; for example is CB1 cannabinoid receptor (CNR1) ; such

as PSG; for example is PSG13' ; such as CPE-receptor; for example is CRH2R; such as OC15 ; for example is TRAIL receptor 2; such as HNMP-1; for example is kidney alpha-2-adrenergic receptor ; such as erythropoietin receptor; for example is chondroitin sulphate proteoglycan versican V1 ; for example is mGluR1 beta; such as CD97; for example is L6; such as NY-ESO-1 ; for example is T-cell receptor alpha delta ; such as ror1 ; for example is ror2; such as SSTR2; for example is VESPR ; such as IgG Fc receptor; for example is glutamate receptor subunit GluRC ; such as HEK2; for example is PVR; such as CEA; for example is CC-chemokine-binding receptor JAB61; such as HER2; for example is HER3 ; such as hypothetical protein FLJ22357 similar to Epidermal growth factor receptor-related protein; for example is putative endothelin receptor type B-like protein; such as GLVR2; for example is P2X4 purinoreceptor; such as FPRL1; for example is Atrial natriuretic peptide clearance receptor; for example is gastrin/CCK-B receptor; such as Neuromedin B receptor; for example is GFRA3; such as GRPR; for example is CDH1; such as CDH2; for example is TGFBR1; such as TGFBR2; for example is TGFBR3; such as precursor of epidermal growth factor receptor.

It is also an objective of the present invention to provide methods of identifying novel drug targets, comprising the steps of i) Providing a binding partner as described in the present invention ii) Identifying potential drug targets capable of associating with said binding partner It is yet another objective of the present invention to provide drug targets identified by the methods described by the present invention.

Furthermore, it is an objective of the present invention to provide targeting complexes comprising: i) A binding partner as described by the present invention; and ii) A bioreactive species wherein the targeting complex is capable of binding a cell surface